

Amendments to the Specification:

Please replace the paragraphs at page 4, line 12 through page 5, line 13 with the following amended paragraphs:

The tobacco mosaic tobamovirus is of particular interest to the instant invention in light of its ability to express genes at high levels in plants. TMV is a member of the tobamovirus group. TMV virions are 300 nm x 18 nm tubes with a 4 nm-diameter hollow canal, and consist of 2140 units of a single structural protein helically wound around a single RNA molecule. The genome is a 6395 base plus-sense RNA. The 5'-end is capped and the 3'-end contains a series of pseudoknots and a tRNA-like structure that will specifically accept histidine. The genomic RNA functions as mRNA for the production of proteins involved in viral replication: a [[126-kDa]] 126 kDa protein that initiates 68 nucleotides from the 5'-terminus and a [[183-kDa]] 183 kDa protein synthesized by readthrough of an amber termination codon approximately 10% of the time (Fig. 1). Only the [[183-kDa]] 183 kDa and [[126-kDa]] 126 kDa viral proteins are required for TMV replication in trans. (Ogawa, T., Watanabe, Y., Meshi, T., and Okada, Y., Virology 185:580-584 (1991)). Additional proteins are translated from subgenomic size mRNA produced during replication (reviewed in Dawson, W. O., Adv. Virus Res. 38:307-342 (1990)). The [[30-kDa]] 30 kDa protein is required for cell-to-cell movement; the [[17.5-kDa]] 17.5 kDa capsid protein is the single viral structural protein. The function of the predicted [[54-kDa]] 54 kDa protein is unknown.

The minimal sequences required in cis for TMV replication are located at the extreme 5' and 3' noncoding regions (replication origins), as determined by analysis of deletion mutants in plant protoplasts (Takamatsu, N., et al., J. Virol. 64:3686-3693 (1990), Takamatsu, N., et al., J. Virol. 65:1619-1622 (1991)). In whole plants, helper-dependent dependent RNA replicons, constructed by deletion of most of the [[126/183-kDa]] 126/183 kDa replication protein sequence and most of the [[30-kDa]] 30 kDa movement protein sequence, are replicated and spread systemically in the presence of wild type TMV (Raffo A. J., and Dawson W. O., Virology 184:277-289 (1991)).

Please replace the paragraphs at page 6, line 12 through page 7, line 29 with the following amended paragraphs:

In the case of TMV, functional [[30-kDa]] 30 kDa movement protein is absolutely required for cell-to-cell movement in whole plants, but can be deleted or inactivated without affecting replication in protoplasts or inoculated leaves (reviewed in Citovsky, V., Zambryski, P., BioEssays 13:373-379 (1991) and Deom, C. M., Lapidot, M., and Beachy, R. N., Cell 69:221-224 (1992)).

A sequence located within the [[30kDa]] 30 kDa movement protein gene of the U1 strain of TMV serves as the origin of assembly. It is at this origin of assembly that the TWV RNA and the viral capsid protein spontaneously aggregate to initiate the assembly of virions (Butler, P. J. G., Mayo, M. A., Molecular architecture and assembly of tobacco mosaic virus particles, The molecular biology of the positive strand RNA viruses. (D. J. Rowlands, M. A. Mayo, and B. W. J. Mahy, eds.), Academic Press, London. pp. 237-257 (1987)). A functional origin of assembly is also required for efficient long distance movement (Saito, T., Yamanaka, K., and Okada, Y., Virology 176:329-336 (1990)). There does not appear to be any additional requirements for packaging. A variety of heterologous sequences can be encapsidated yielding rod-shaped virions whose lengths are proportional to the size of the RNA molecule containing the origin of assembly (Dawson, W. O. et al., Virology 172:285-292 (1989)).

Construction of plant RNA viruses for the introduction and expression of foreign genes in plants is demonstrated by French, R., et al., Science 231:1294-1297 (1986); Takamatsu, N., et al., EMBO J. 6:307-311 (1987); Ahlquist, P., et al., Viral Vectors, Cold Spring Harbor Laboratory, New York, 183-189 (1988); Dawson, W. O., et al., Phytopathology 78:783-789 (1988); Dawson, W. O., et al., Virology 172:285-292 (1989); Cassidy, B., and Nelson, R., Phytopathology 80:1037 (1990);

Joshi, R. L., et al., EMBO J. 9:2663-2669 (1990); Jupin, I., et al., Virology 178:273-280 (1990); Takamatsu, N., et al., FEBS Letters 269:73-76 (1990); ~~Japanese~~ Japanese Published Application No. 63-14693 (1988); European Patent Application No. 067,553; and European Patent Application No. 194,809, European Patent Application No. 278,667. Most of the viral vectors constructed in these references were not shown to be capable of systemic movement in whole plants. Rather, gene expression has only been confirmed in inoculated leaves. In other cases, systemic movement and expression of the foreign gene by the viral vector was accompanied by rapid loss of the foreign gene sequence (Dawson, W. O., et al., Virology 172:285 (1989)).

Please replace the paragraph at page 14, lines 7-21 with the following amended paragraph:

In a further embodiment, the replicon codes for a functional movement protein such as the ~~[[30kDa]]~~ 30 kDa TMV movement protein. The helper virus used in this embodiment does not possess a functional movement protein. Thus, the helper virus is dependent on the replicon for movement functionality. Movement proteins are necessary for cell to cell movement in plants. By placing a functional movement protein sequence on the replicon and either deactivating or deleting the same sequence on the helper virus or by using a host species with helper virus encoded movement protein incompatibility, the helper virus's dependency on the replicon enables systemic infection of the whole plant with the viral replicon plus helper virus.

Please replace the paragraph at page 16, lines 4-14 with the following amended paragraph:

The foreign gene product coded for and expressed by the replicon can be a very wide variety of RNA or proteins products and include, for example, antisense and ribozyme RNA, regulatory enzymes, and structural, regulatory and therapeutic proteins that may be expressed in their native form or as gene fusions. Typical therapeutic proteins include members of the interleukin family of proteins and colony stimulating factors (CSFs) such as CSF-G (granulocyte colony stimulating factor), CSF-GM (granulocyte-macrophage colony stimulating factor) and CSF-M (macrophage colony stimulating factor). It is understood, however, that any therapeutic protein can be coded for and expressed in the instant invention.

Please replace the paragraphs at page 17, line 15 through page 18, line 33 with the following amended paragraphs:

Construction of a transgene derived from TMV is set forth herein. The wild type TMV genome is set forth in FIG. 1. The construction of DNA plasmids containing the 5' replication origin fused to the CaMV 35S promoter are described in (Ow, D. W., et al., Science 234:856-859 (1986)) and the 3' replication origin fused to a ribozyme termination region are described by Turpen, T. H., Ph.D. ~~Dissertation~~ Dissertation, University of California, Riverside, pp. 88-105 (1992).

The substitution of the coat protein gene for the coding sequence of CAT is described in Dawson, et al., Phytopathol. 78:783-789 (1988). These previously disclosed plasmids, pBGC43, pBGC44, pBGC75 (Turpen, T. H., Ph.D. ~~Dissertation~~ Dissertation, University of California, Riverside, pp. 88-105 (1992)) and pTMVS3CAT28 (Dawson, et al., Phytopathol. 78:783-789 (1988)) are used as precursors for the construction of the desired transgene for synthesis of replicon RNA (FIG. 5).

In this construction, it is desired to place the ~~[[30-kDa]]~~ 30 kDa movement protein gene at precisely the same position as the replicase gene (relative to 5' replication origin in the wild type TMV genome, See Figure 5). To accomplish this, a NdeI site is introduced at the start codon of each gene by PCR-based mutagenesis using synthetic primers and unique adjacent cloning sites. A 270 bp mutagenesis product containing the internal NdeI site from the PCR primer is subcloned using the

EcoRV site in the cauliflower mosaic virus 35S promoter and the HindIII site in the [[30-kDa]] 30 kDa protein gene. The ligation product is then sequence verified.

The 3' segment of the replicon, containing the CAT gene will be placed adjacent to the 3'-ribozyme as a HindIII-NsiI fragment from the transient TMV vector pTMVS3CAT28 (FIG. 5). In the final cloning step, the 5' portion of the transgene and the 3' portion will be subcloned into the unique BamHI site of the plant transformation vector pAP2034 (Velton and Schell, NAR 13:6981-6998 (1985) as a BglII-BamHI fragment described previously (Turpen, T. H., Ph.D. ~~Dissertation~~ Dissertation, University of California, Riverside, pp. 88-132 (1992)). The sequence of the replicon RNA, produced by host transcription, RNA processing, and replication in the presence of a helper virus, is given in FIG. 6. Thus, the foreign gene (CAT) is placed on a RNA viral replicon, under control of the coat protein subgenomic promoter for messenger RNA synthesis (located at the 3' end of the movement protein gene).

Please replace the paragraph at page 20, line 18 through page 21, line 21 with the following amended paragraph:

The sequence of the replicon RNA, produced by host transcription, RNA processing, and replication in the presence of a helper virus, is given in Fig. 6. Tobamoviruses with mutations or naturally occurring variation in the [[30-kDa]] 30 kDa protein gene are deficient in cell-to-cell movement on specific host species. Transgenic plants or alternate hosts can complement this defect. It will be appreciated to those skilled in the art that there are numerous methods of producing helper tobamoviruses by genetic engineering or by mutagenesis in addition to those helper variants or host species combinations occurring naturally. Likewise, methods for producing transgenic plants which express 30 kDa protein and which complement defective 30 kDa containing viruses have been published. For example, movement deficient helper viruses can be synthesized by transcription of TMV with known mutations for the production of RNA inoculum. Transgenic plants expressing the [[30-kDa]] 30 kDa protein complement this defect (Deom, C. M., et al., Science 237:389-394 (1987)). Therefore, large quantities of a helper virus can be propagated. In one embodiment of this invention, a [[30-kDa]] 30 kDa protein frameshift mutant, having a single base pair deletion at position 4931 thereby creating a EcoRV site in the cDNA, is used as helper virus. Transgenic tobacco (about 100 plants) are regenerated containing this replicon transgene construction and assayed for CAT activity in the presence and absence of helper viruses using procedures described (Shaw, W. V., Chloramphenicol acetyltransferase from chloramphenicol-resistant bacteria, Methods in Enzymology, Vol. 53, (S. Fleischer and L. Packer, eds.), pp. 737-755 (1975)). 200 mg of leaf tissue is macerated in assay buffer followed by the addition of 0.5 mM acetyl CoA and 0.1 uCi [¹⁴C]chloramphenicol, incubation for 45 min at 37° C, extraction, resolution by thin-layer chromatography, and autoradiography.